Clinical research

Pre-procedural plasma levels of C-reactive protein and interleukin-6 do not predict late coronary angiographic restenosis after elective stenting

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Aims Inflammatory markers may serve as an important prognostic predictor in patients with coronary heart diseases. In patients undergoing coronary interventions, it has been shown that baseline C-reactive protein (CRP) could predict late clinical restenosis. Only a few small studies have examined the possible relationship with angiographic restenosis. In patients with stable angina pectoris, we examined whether baseline CRP and IL-6 predict late coronary angiographic restenosis after stenting.

Methods and Results Pre-procedural plasma levels of CRP and IL-6 were measured in 216 patients with stable angina pectoris undergoing elective coronary stenting. Angiographic follow-up was performed in all patients at 6 months. Baseline CRP levels were $6.15 \pm 0.78$ mg/L versus $5.24 \pm 1.17$ mg/L in the patent and restenosis groups, respectively ($P = 0.64$). IL-6 levels were $0.46 \pm 0.03$ ng/L versus $0.40 \pm 0.07$ ng/L in the patent and restenosis groups, respectively ($P = 0.50$). CRP levels were obtained again at the time of angiographic follow-up and were found to be similar in both groups ($2.89 \pm 0.29$ mg/L versus $2.61 \pm 0.63$ mg/L, $P = 0.72$). Moreover, in a sub-group of 43 patients, serial blood samples were obtained at several time points after the procedure up to 6 months. Both CRP and IL-6 plasma levels increased significantly in response to the procedure. CRP levels peaked at 3 days ($11.27 \pm 1.53$ mg/L versus $4.26 \pm 0.72$ mg/L at baseline, $P < 0.0001$). IL-6 levels reached maximum values after 24 h ($1.08 \pm 0.14$ ng/L versus $0.53 \pm 0.08$ ng/L at baseline, $P < 0.0001$). However, in this sub-group of patients, neither peak CRP nor IL-6 levels were found to predict late angiographic restenosis.
Introduction

Inflammation plays an important role in the pathogenesis of atherosclerosis and restenosis. Several markers are known to represent the systemic inflammatory status, among them are C-reactive protein (CRP) and IL-6. Several studies have shown that high plasma levels of inflammatory markers predict the extent of atherosclerosis and subsequent coronary events among healthy individuals. CRP has been shown to be an excellent prognostic marker in patients with stable angina, unstable angina, and acute myocardial infarction. Arterial injury caused by coronary intervention results in the induction of several inflammatory components. Several clinical studies have shown that the baseline levels of systemic inflammatory markers such as CRP are independent prognostic indicators for subsequent cardiac events and clinical restenosis. Only a few studies have examined the relation between pre-procedural levels of CRP and IL-6 and late, 6-month, angiographic restenosis.

The aim of this study was to assess the predictive value of pre-procedural levels of CRP and IL-6 in 6-month angiographic restenosis in a convenience cohort of patients undergoing coronary stenting. Additionally, we assessed the effects of the intervention on post-procedural changes in these marker levels.

Methods

Patients

The study population consisted of 216 patients from three coronary stent studies, which performed rigorous quantitative angiography and protocol-specific 6-month follow-up coronary angiography. These studies were the MedStent registry (in press manuscript), the DISTINCT (BiodivYsio® Stent In Randomized Control Trial) trial, and a prospective registry of patients undergoing coronary stenting at St. Michael’s Hospital (SMH). The SMH group (n = 43) was also included in a sub-study where serial blood samples were taken before and at several time points after the procedure. All patients, in all three studies, underwent elective coronary stenting for stable angina.

The MedStent Registry consisted of 120 elective patients treated with the coronary MedStent in 6 centres in Canada between April 2000 and May 2002. A subgroup of 76 consecutive patients within this study had plasma samples drawn pre-procedure and at the time of the 6-month angiographic follow-up. All patients had symptomatic coronary artery disease and a single de novo lesion in a native coronary artery with a diameter stenosis greater than 50%. Patients were included with lesion length ≤ 14 mm and reference diameter ≥ 3 mm based on visual assessment. Exclusion criteria were: unstable angina with ECG changes despite heparin within the past 24 h, myocardial infarction within the past 7 days, total occlusion, angiographic evidence of intra-coronary thrombosis, aorto-ostial lesions, lesion at a bifurcation where the side branch diameter was greater than or equal to 2.5 mm, by-pass graft stenosis, previous angioplasty at the site of intervention, and creatinine > 180 μmol/L. Blood specimens were available for 76 patients, pre-procedure.

All patients were pre-treated with aspirin 325 mg and either 250 mg ticlopidine or 300 mg clopidogrel. Unfractionated heparin was given during the procedure in order to maintain an activated clotting time of > 220 seconds. Ticlopidine 250 mg bid or clopidogrel 75 mg daily was continued for 2–4 weeks, at the physician’s discretion. Angiography was performed in at least two orthogonal projections after intra-coronary administration of nitroglycerin. The procedure was considered successful when the residual % DS in the dilated segment was < 50% immediately after angioplasty and no major complications ensued (in-hospital death, urgent bypass surgery, re-do percutaneous coronary intervention, or myocardial infarction). Angiographic measurements were analyzed offline with the Cardiac Measurement System (MEDIS, Medical Imaging Systems, Leiden, The Netherlands) using techniques that have been described in detail elsewhere. All patients were scheduled for an elective 6 month angiographic follow-up. When repeat re-vascularisation of the treated segment was performed before protocol-specified 6-month angiography, the clinical angiography triggering repeat re-vascularisation was defined as follow-up angiography. If follow-up angiography was performed at less than 4 months after the stent placement, and no second intervention was performed, patients were asked to undergo repeat angiography at 6 months. Angiographic in-stent restenosis was defined as DS > 50% within or at the edges of the stent.

Measurement of CRP and IL-6

Venous blood was drawn at baseline (pre-procedure) and at the time of follow-up angiography. CRP levels were measured at baseline and after 6 months, while IL-6 levels were measured only at baseline. In the SMH cohort of 43 patients, venous blood was drawn at baseline and at several time points after the procedure (6 h, 24 h, 3 days, 7 days, 1 month, 3 months and 6 months post stenting). Plasma samples were stored at −20 °C.

Conclusions Coronary stenting is associated with transient increases in both CRP and IL-6 levels. However, pre-procedural CRP and IL-6 levels do not predict late coronary angiographic restenosis.

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until assays were performed. Plasma CRP concentration was determined using the Behring BN100 and the N high-sensitivity CRP reagent (Dade-Behring, Mississauga, ON, Canada). IL-6 was measured using the biosource ELISA (Human IL-6 US UltraSensi-

Statistical analysis

For continuous data the mean ± SEM were calculated. Restenosis was defined by a binary approach of taking the commonly used cut-off of >50% DS at angiographic follow-up. To determine whether data from the three studies could be pooled, baseline values of the inflammatory markers were compared between studies using analysis of variance (ANOVA). Similarly, the restenosis rates between the three studies were also compared by ANOVA. For pooled data, differences in the pre-procedural CRP mean values between restenosis (DS > 50% at follow-up) and patent (DS < 50% at follow up) groups were assessed by a two-sided Student t test. Similar analyses were performed for pre-procedural IL-6 and 6-month CRP values.

The relationship between baseline levels of inflammatory markers and absolute late lumen loss was evaluated in two different ways. First, baseline levels of CRP and IL-6 were assessed against absolute late lumen loss using an ANOVA F test. Second, the baseline markers of CRP and IL-6 were divided into tertiles and assessed against late lumen loss using ANOVA F test.

To determine the predictive value of baseline levels of CRP and IL-6 and other possible clinical variables, a multivariate stepwise regression analysis (SAS software, version 8.2) was performed on baseline variables including study (MedStent, DISTINCT, and SMH), diabetes mellitus, LAD intervention, lesion length, reference diameter, minimal luminal diameter (MLD) pre-procedure, MLD immediately post-procedure, and baseline CRP and IL-6, regressed against angiographic restenosis at 6 months. A P-value of 0.15 was used for inclusion of variables in the model. To further assess whether the relationship between angiographic restenosis and baseline levels of inflammatory markers was consistent across the three studies, a logistic regression analysis was performed. Restenosis was regressed against study, baseline levels of inflammatory markers and their interaction.

In the subgroup of 43 patients with serial measurements of inflammatory markers, differences between baseline and each time point were calculated for each patient and the mean differences were compared to zero using ANOVA with repeated measures. A value of P < 0.05 was considered statistically significant.

Results

Baseline demographics and baseline and follow-up angiographic characteristics are summarised in Table 1. There were no significant differences in baseline levels of CRP and IL-6 or restenosis rates between the three studies indicating that the data could be pooled. Overall, angiographic restenosis (DS > 50%) was diagnosed in 15% of patients.

In the restenosis group, baseline CRP levels were 5.24 ± 1.17 mg/L compared to 6.15 ± 0.78 mg/L in the patent group (P = 0.64) (Fig. 1(a)). At 6-month angiographic follow-up, CRP levels in the restenosis and patients groups were 2.61 ± 0.63 and 2.89 ± 0.29 mg/L, respectively (P = 0.72) (Fig. 1(b)). Baseline IL-6 levels in the restenosis and patent group were 0.40 ± 0.07 and 0.46 ± 0.03 ng/L, respectively (P = 0.50) (Fig. 1(c)). As illustrated in Fig. 2, there was no relationship between baseline CRP or IL-6 and 6-month in-stent late loss. Furthermore, no differences in 6-month in-stent late loss were found among tertiles of baseline plasma levels of CRP and IL-6 (Fig. 3).

The step-wise multivariate regression analysis showed that none of the baseline variables predicted restenosis. The logistic regression analysis of baseline inflammatory markers, using %DS as a binary variable, showed no significant differences in the mean values of either CRP

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics</th>
<th>Distinct study (n = 97)</th>
<th>MedStent registry (n = 76)</th>
<th>SMH study (n = 43)</th>
<th>Total (n = 216)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>57 ± 10</td>
<td>59 ± 10</td>
<td>57 ± 9</td>
<td>58 ± 10</td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>72</td>
<td>70</td>
<td>77</td>
<td>72</td>
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<tr>
<td><strong>Diabetes (%)</strong></td>
<td>14</td>
<td>18</td>
<td>23</td>
<td>17</td>
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<tr>
<td><strong>Hypertension (%)</strong></td>
<td>39</td>
<td>47</td>
<td>31</td>
<td>40</td>
</tr>
<tr>
<td><strong>Current smoking (%)</strong></td>
<td>27</td>
<td>25</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td><strong>Previous MI (%)</strong></td>
<td>39</td>
<td>43</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td><strong>Target vessel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD (%)</td>
<td>33</td>
<td>46</td>
<td>62</td>
<td>42</td>
</tr>
<tr>
<td>RCA (%)</td>
<td>54</td>
<td>38</td>
<td>21</td>
<td>43</td>
</tr>
<tr>
<td>LCx (%)</td>
<td>13</td>
<td>16</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Reference diameter (mm)</td>
<td>3.23 ± 0.05</td>
<td>3.26 ± 0.05</td>
<td>2.87 ± 0.09</td>
<td>3.17 ± 0.03</td>
</tr>
<tr>
<td>Lesion length (mm)</td>
<td>14.51 ± 0.66</td>
<td>10.48 ± 0.36</td>
<td>9.94 ± 0.50</td>
<td>12.18 ± 0.34</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>0.65 ± 0.05</td>
<td>0.68 ± 0.04</td>
<td>0.81 ± 0.05</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>Diameter stenosis (%)</td>
<td>85 ± 1</td>
<td>80 ± 1</td>
<td>71 ± 2</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>Net gain</td>
<td>2.84 ± 0.08</td>
<td>2.58 ± 0.05</td>
<td>1.91 ± 0.09</td>
<td>2.57 ± 0.04</td>
</tr>
<tr>
<td>6-Month late loss</td>
<td>0.87 ± 0.10</td>
<td>1.16 ± 0.08</td>
<td>0.60 ± 0.11</td>
<td>0.92 ± 0.06</td>
</tr>
<tr>
<td>6-Month binary restenosis (%)</td>
<td>10.3</td>
<td>19.7</td>
<td>16.3</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM, unless indicated otherwise.

MI, myocardial infarction; LAD, left anterior descending; RCA, right coronary artery; LCx, left circumflex; MLD, minimal luminal diameter.
There were no significant interactions between the studies and any of the inflammatory markers ($P = 0.81, P = 0.67$, and $P = 0.46$ for pre-procedural IL-6, pre-procedural CRP, and 6-month CRP, respectively).

In the subgroup of 43 patients in whom serial blood samples were taken, both CRP and IL-6 significantly increased after the procedure. The CRP plasma level peaked at 3 days, $11.27 \pm 1.53$ mg/L, compared to the baseline level of $4.26 \pm 0.72$ mg/L ($P < 0.0001$) and was also significant at 1 day, $7.85 \pm 0.99$ mg/L ($P < 0.0001$). At 7 days after the procedure, plasma levels had returned to baseline (Fig. 4(a)). IL-6 plasma levels reached an earlier peak at 24 h post-procedure, $1.08 \pm 0.14$ ng/L, compared to baseline level of $0.53 \pm 0.08$ ng/L ($P < 0.0001$), and then returned to baseline at 7 days (Fig. 4(b)).

**Discussion**

The major finding of this study is that pre-procedural plasma levels of CRP and IL-6 had no relationship to 6-month angiographic restenosis in patients with stable angina pectoris undergoing elective coronary stenting for de novo, single coronary artery lesions. Moreover, we have also demonstrated that while stenting causes transient elevation in both CRP and IL-6, the magnitude of this elevation does not distinguish patients who develop angiographic restenosis from those who do not.

In vitro and animal experiments have suggested that inflammation plays an important role in the pathogenesis of intimal hyperplasia after arterial injury.$^{9,21}$ In a rabbit model of balloon angioplasty, it was shown that activation of the transcription factor nuclear factor-κB (NF-κB), which is the common factor driving the inflammatory
response, significantly contributes to late lumen loss. Other animal studies have demonstrated that after stenting, a particularly brisk early inflammatory response is induced with abundant surface adherent monocytes and granulocytes. Days and weeks later, macrophages invade the forming neo-intima and are observed clustering around stent struts.

Several clinical studies have shown that pre-procedural inflammatory status as manifested by CRP levels is a strong prognostic predictor of mortality and subsequent cardiac events (e.g., clinical restenosis). Buffon et al. reported that baseline CRP was the most powerful predictor of clinical restenosis after balloon angioplasty, even in patients with a good immediate angiographic result (relative risk = 12.2). In a study of 501 patients the incidence of death, or myocardial infarction, during a 2-year follow-up, after elective coronary angioplasty, was 3.9-fold higher in patients with increased baseline CRP levels. Even when adjusted to conventional coronary disease risk factors and procedural characteristics, baseline CRP levels remained independently predictive of adverse events, with the highest quartile of CRP associated with an odds ratio for excess 30-day death or myocardial infarction of 3.68. However, a different study of 415 patients undergoing PCI for stable and unstable angina and acute myocardial infarction failed to show a significant prognostic predictive value of CRP.

In contrast to the majority of studies, which examined the relationship between baseline CRP and late clinical events, only a few studies have assessed the relationship between CRP plasma levels and late angiographic restenosis. Initially, Pietersma et al. showed that the production of IL-1β by stimulated monocytes is associated with late lumen loss. However, they failed to show any relation between baseline CRP and late lumen loss. Opposite results were demonstrated in a small study by Gottauner-Wolf et al., who examined the relationship between plasma levels of CRP after the procedure with late angiographic restenosis. They suggested that persistently elevated CRP plasma levels at 96 h after stent implantation may reflect a prolonged inflammatory response and contribute to the development of late restenosis. In a different study of 75 patients undergoing balloon angioplasty or directional atherectomy, the authors failed to find an association between restenosis and elevated CRP levels. The largest study to date with angiographic follow-up at 6 months after coronary stenting has been reported by Walter et al. In patients with mainly unstable coronary syndromes, including acute MI (with 86% follow-up angiography), the authors showed that high baseline levels of CRP were associated with increased rate of restenosis. Our study is different from the report by Walter et al., in the following aspects: First, Walter et al., used the turbidimetric method and not the recently recommended high-sensi-

![Fig. 4 Serial measurements of CRP (panel a) and IL-6 (panel b) after stenting. Values are mean ± SEM.](image-url)
tive CRP assay. Secondly, their study consisted of a mixed group of patients including patients with acute coronary syndromes (41% of patients with very high CRP had acute MI), while our study consisted of a homogeneous group of patients with stable angina who underwent elective stenting.

IL-6 plasma levels have also been shown to be an important prognostic predictor in healthy populations as well as in various clinical settings such as unstable angina. A recent study among elderly patients has shown that a single determination of serum inflammatory markers, particularly elevated IL-6, was associated with an increased risk of CHF. With regards to arterial repair, IL-6 has been shown to stimulate the growth of vascular smooth muscle cells. As mentioned before, Pietersma et al. have demonstrated no correlation between baseline IL-6 and late lumen loss in a small study of 32 patients undergoing balloon angioplasty. In contrast, a Japanese study of 32 patients showed that the extent of increase in the plasma concentration of IL-6 obtained from the coronary sinus before and immediately after the coronary intervention was predictive of late lumen loss index 6 months after the procedure. A different small study of 20 patients showed that IL-6 levels post-procedure were increased in patients with restenosis following balloon angioplasty.

Our current study is the first to show no relationship between pre-procedural levels of either CRP or IL-6 and late angiographic restenosis in a homogeneous and large cohort of patients undergoing contemporary coronary stenting for stable angina pectoris. It appears clear from these data that high baseline levels of systemic inflammatory markers, at least the markers tested in the present study, do not indicate increased local susceptibility to restenosis at the site of coronary intervention. Other studies have suggested that elevated levels of CRP and IL-6 may indicate, however, more advanced and unstable generalised atherosclerotic disease. This difference underscores other known pathophysiological, pathological, angiographic and clinical distinctions between post-PCI restenosis and spontaneous atherothrombotic coronary narrowing.

Study limitations

First, our study group consisted of patients from three separate studies. However, the inclusion criteria, the range of inflammatory marker levels, and restenosis rates were similar in all three groups. Despite minor differences in the design and objectives of the three clinical studies, there were no significant differences between studies in terms of the relationship between inflammatory markers and %DS. Thus, the data from all studies could be combined. Secondly, our study consisted of low-risk patients with stable angina and may not be applicable to different subsets of patients such as acute coronary syndromes. Furthermore, it is possible that aspirin and statins or other successful risk factor interventions initiated before the coronary intervention may have altered the plasma levels of the inflammatory markers.

Clinical implication

Although many clinical studies in various clinical settings have suggested that CRP (or IL-6) may be a good prognostic tool to predict future cardiac events, our findings do not support any relationship between angiographic restenosis and plasma levels of CRP and IL-6 in patients with stable angina undergoing elective coronary stenting.

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References


